PATENT

8759-2-1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
KEVIN W. MOORE et al.	Examiner: H. Schain
Serial No. 558,551	Art Unit: 153
Filed: December 5, 1983	
For: HYBRID DNA PREPARED BINDING COMPOSITION	Declaration Under 37 C.F.R. 1.132
	San Francisco, CA 94105

Hon. Commissioner of Patents and Trademarks
Washington D.C. 20231

Sir:

I, Kevin W. Moore, do hereby declare as follows:

I am a coinventor of the above-identified patent application and, as of the filing date of the parent application of the subject application and presently, am employed as a research scientist at DNAX Research Institute of Molecular and Cellular Biology, Inc.

I have a Ph.D. in chemistry from the California Institute of Technology and remained at this institution to complete a National Institutes of Health Postdoctoral Fellowship in biology. I am an author of numerous published scientific articles in the fields of biochemistry, microbiology, and immunology. (See attached C.V.) KWM

The subject invention concerns specific binding compositions ("rFv") which integrally contain two polypeptide chains. Each of these polypeptide chains is at least a portion of the variable region of a heavy or light chain of an immunoglobulin that has a binding specificity for a selected ligand. Further, each polypeptide of the subject invention is produced by means of recombinant DNA technology whereby the DNA coding for the rFv polypeptides

is lacking the sequence coding for the natively associated constant region.

Until the subject invention, no procedure existed for the direct synthesis of polypeptides consisting of either a portion of or the entire variable region of an immunoglobulin heavy or light chain. Further, the production of immunoglobulin polypeptide fragments by the traditional method of enzyme cleavage often resulted in compositions containing extraneous materials that were extremely difficult to remove by conventional purification techniques. Such impurities severely restrict therapeutic usefulness of enzymatically generated polypeptide fragments. Additionally, such enzymatically produced fragments typically contain portions of the constant region in addition to the variable region. With the subject invention, the opportunity now exists to produce various immunoglobulin-like binding compositions in both high yield and purity.

The rFv compositions are smaller than the corresponding immunoglobulins, but still offer high specificity and affinity for desired ligands. As stated in the subject application, these smaller rFv species provide for numerous benefits over the native immunoglobulins and include; shorter residence times in mammalian hosts, minimized size of final bound complex, higher packing density for active binding sites, controlled binding affinity and specificity, and multiple binding sites.

I have reviewed the following references and have concluded that neither individually nor in combination do they identically describe the subject invention nor would they lead to the subject invention without extensive experimentation.

Sharon et al., Biochemistry, 15:1591-1594 (1976);

Rosemblatt et al., Biochemistry, 17:3877-3882 (1978);

Pawlowski et al., J. Biol. Chem., 250:2135-2142 (1975);

Zakut et al., Nucleic Acids Res., 8:3591-3601 (1980);

Seidman et al., Nature, 280:370-375 (1979);

Early et al., Cell, 19:981-992 (1980);

Amster et al., Nucleic Acids. Res., 8:2055-2065 (1980);

Ptashne et al., U.S. Pat. No. 4,332,892;

Ehrlich et al., U.S. Pat. No. 4,355,023;

Auditore-Hargreaves, U.S. Pat. No. 4,470,925.

I, as one skilled in the biotechnology art, base this opinion in part on the fact that recombinant DNA technology is not employed in any of these references to produce light or heavy chain polypeptide fragments that retain their ability to form active binding species. Therefore, to apply recombinant DNA technology to produce the rFv species, as was done in the subject application, would have necessitated extensive and prolonged experimentation with no guarantee of success.

For purposes of discussion, an expression construct of the subject invention includes an expression vector with the necessary control elements for expression of a nucleotide sequence in a selected host, wherein the nucleotide sequence (such as cDNA) codes for at least a portion of a variable region of a selected heavy or light immunoglobulin chain and any desired leader region (but without nucleotide sequence coding for the constant region). With this in mind, it is my considered opinion that the subject application presents more than sufficient details for a molecular biologist, or one of similar scientific training, to produce the subject invention's expression constructs. Further, it is my firm belief that by employing

the subject application's procedures, such a scientist could use the expression constructs in a selected foreign host to produce the desired variable region polypeptide chains. Additionally, once such variable region polypeptides are produced, the subject application adequately describes to such a scientist how such chains could be isolated and combined (renatured) to form the subject rFv in active pure form.

As can be seen in the subject application beginning at page 5, line 16 and continuing to page 16, line 23, and from page 19, line 10 to page 41, line 12, an extremely detailed description is given concerning the exact steps required to produce the expression constructs employed for the production of the subject invention polypeptide chains. Precise experimental procedures are stated from page 19, line 10, to page 41, line 12, for the production of the expression constructs and include required volumes, buffers, concentrations, reaction times, temperatures, reagents, cell lines, and the like.

All of the essential elements for proper transcription and translation are fully disclosed and include in the expression construct an appropriate promoter, operator, attenuator, or activator, a region of cDNA coding for the variable region of the desired polypeptide and any associated leader region, a ribosomal binding site, initiation codon, and termination region. No excessive or undue experimentation would be required to follow these thoroughly detailed steps.

There is no doubt that the various steps discussed in the subject application describe functional procedures. The "genetic engineering" industry at large has come to rely on and practice essentially these same procedures to produce

a variety of polypeptides and proteins. Such procedures as those described in the subject application for the production of rFv and those employed generally in the field have produced marketed polypeptide products such as human growth factor and the two chain protein insulin.

Concerning the transcription and translation of the rFv polypeptide chains, the subject application provides the required details essential for the expression of the relevant variable portions of the light and heavy chains in a foreign host. Introduction of the expression constructs into the appropriate host is adequately described on page 16, line 24 to page 17, line 17 and page 41, lines 12 to 23 of the subject application. Further, as can be seen in two articles, Wood et al., Nature, 314:446-449 (1985) (Exhibit A) and Boss et al., Nucleic Acids Res., 12:3791-3806 (1984) (Exhibit B), the application of techniques substantially similar to those of the subject application can result in the expression of immunoglobulin polypeptide chains. Once expression constructs containing intact immunoglobulin light or heavy chain cDNA coding regions were introduced into either an E. coli host (Boss et al.) or a yeast host (Wood et al.), these hosts did express the desired immunoglobulin polypeptides. Substantially similar procedures for expression in a foreign host were discussed in the subject application. Although both of these articles refer to expression of intact immunoglobulin polypeptide chains, similar results for expression of the subject invention's shorter chains are reasonably expected. Clearly then, the procedures describing the expression of the subject expression constructs to produce the rFv variable region chains are enabling to a skilled molecular biologist.

The isolation of the rFv variable region heavy and light immunoglobulin chains by any one of a variety of possible purification techniques (e.g., gel electrophoresis, fractional precipitation, affinity chromatography, high performance liquid chromatography, and the like) is fully disclosed in the subject application at page 17, lines 5 to 17. In particular, the purification technique of immunosorbent affinity chromatography is presented in the subject application for non-secreted product at page 17, lines 35ff and page 41, lines 16ff and for secreted product at page 17, lines 18ff. Both the Wood et al. and Boss et al. articles relied on such purification methods to isolate their polypeptide products. Additionally, the specific technique of immunosorbent affinity chromatography discussed in the subject application is reliable and well-known in the art (see, for enzymatically produced variable region purification, Ehrlich, et al., U.S. Patent No. 4,355,023).

As is evidenced by the papers of Boss et al. and Wood et al., functionally active antibodies may be produced by renaturing the appropriate dissociated heavy and light chains by standard dialysis procedures. The subject application adequately discloses suitable procedures for this renaturation process and for the final rFv purification, such as by ligand-specific affinity chromatography at page 18, lines 3ff and page 41, line 32 to page 42, line 13. The subject application differs from the Wood et al. and Boss et al. references in the use of the shorter variable region chains versus the intact constant/variable region chains. However, the renaturation process by dialysis would be fundamentally the same regardless of the size of the polypeptide, so long as the polypeptide is retained within the dialysis membrane. Evidence for the position that

renaturation of immunoglobulin fragments containing the variable region is feasible and practiced as found in Ehrlich et al. (U.S. Pat. No. 4,355,023), where enzymatically produced fragments, composed essentially of polyclonal immunoglobulin variable regions, were shown to renature into active binding species.

Therefore, from the above analysis, it is abundantly clear that the subject application specifically relates detailed and enabling procedures for the expression (transcription and translation), purification and renaturation of the active binding species of the present invention comprising the variable regions of immunoglobulin heavy and light polypeptide chains produced by recombinant DNA techniques.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated April 10, 1986

Kevin W Moore

Kevin W. Moore, Ph.D.

CURRICULUM VITAE

PERSONAL DATA

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EDUCATION

California Institute of Technology
Division of Chemistry and Chemical Engineering
Ph.D. (Chemistry) 1979

Thesis: Mechanism of Action of Adenosylcobalamin
Advisor: Dr. John H. Richards

Princeton University, Princeton, New Jersey
A.B. (Chemistry) 1974

Magna cum laude in Chemistry, 1974;
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Thesis: Physical Chemistry of Proteins
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National Merit Scholar, 1970-1974

EMPLOYMENT

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Research Fellow (Damon Runyon/Walter Winchell Cancer Fund), Division of Biology, California Institute of Technology, 1979-1980.

Graduate Teaching Assistant, California Institute of Technology, Division of Chemistry and Chemical Engineering, 1974-1979.

Freelance translator of Soviet scientific journals for Allerton Press and Plenum Press (Consultants Bureau), 1972-1974.

Research Assistant, Department of Chemistry, Princeton University
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PUBLICATIONS:

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46423349-LK

The following is the nucleotide sequence of the heavy chain variable region of myeloma S107, with the leader, variable region and constant region separated by gaps, and only the first nine amino acids of the constant region depicted. (Early et al. (1980), Cell. 19:981-992).